

IT IS CLAIMED:

1. A method of transforming rice plants with one or more heterologous nucleic acid coding sequences capable of producing heterologous proteins in the rice, under selected induction conditions, comprising
5 cotransforming rice callus cells with a set of two or more expression cassettes, said set comprising:

(a) a chimeric selectable marker expression cassette having, operatively linked in sequence in a 5' to 3' direction, (i) a transcriptional regulatory region which expresses in transformed callus cells at a significantly higher level than in seed tissue, and hybridizes under high stringency conditions with the rice β -glucanase gene (Gns9) promoter identified by SEQ ID NO:1; (ii) a phosphinothricin acetyltransferase- coding sequence and (iii) a 3' untranslated terminator region; and

(b) at least one heterologous gene expression cassette, having operatively linked in sequence in a 5' to 3' direction, (i) a transcriptional regulatory region that is expressed, induced or inducible in plant seeds, (ii) a first DNA sequence encoding a selected heterologous protein, and (iii) a 3' untranslated terminator region, wherein the transcriptional regulatory region in said heterologous-gene expression cassette is induced during seed maturation or seed germination;

20 (i) culturing the callus cells in the presence of a selection agent effective to block growth of callus cells in the absence of expression of the phosphinothricin acetyltransferase-encoding nucleic acid sequence; selecting those callus cells that express the phosphinothricin acetyltransferase enzyme, as evidenced by their growth in the presence of the selection agent; and

25 (ii) regenerating the selected callus cells into transgenic plants under non-selection conditions.

2. A rice plant transformed by the method of claim 1.

30 3. A plant transformation expression cassette for transforming rice plant cells with a chimeric selectable marker gene, said cassette comprising, in a 5' to 3' direction:
(i) a transcriptional regulatory region comprising a sequence which hybridizes under high stringency conditions with the rice β -glucanase gene promoter identified by SEQ ID NO:1, and which expresses in callus cells at a significantly higher level than in a selected target tissue,

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(ii) a phosphinothricin acetyltransferase encoding selectable marker coding sequence, and

(iii) a 3' untranslated terminator region.

5 4. A plant transformation expression cassette according to claim 3, wherein the transcriptional regulatory region in the selectable marker gene is the Gns9 promoter identified by SEQ ID NO:1 or an operative portion thereof, said portion sufficient to promote expression in transformed callus cells at a significantly higher level than in a selected target tissue.

10 5. A rice plant produced by regenerating a plant cell transformed with the expression cassette of claim 4.

15 6. Transgenic rice seed comprising:

a chimeric selectable marker gene including a phosphinothricin acetyltransferase selectable marker coding sequence under the control of a transcriptional regulatory region that is induced in callus [plant] tissue at a significantly higher level than in seed tissue and hybridizes under high stringency conditions with the rice β -glucanase gene (Gns9) promoter identified by SEQ ID NO:1, and

20 a heterologous protein coding sequence under the control of a transcriptional regulatory region that is induced or inducible during seed maturation or germination.

25 7. The transgenic rice seed of claim 6, wherein said chimeric selectable marker gene has the sequence identified by SEQ ID NO:4.

30 8. A method of transforming wheat plants with one or more heterologous nucleic acid coding sequences capable of producing heterologous proteins in the wheat, under selected induction conditions, comprising

35 cotransforming wheat immature embryo cells with a set of two or more expression cassettes, said set comprising:

(a) a chimeric selectable marker expression cassette having, operatively linked in sequence in a 5' to 3' direction, (i) a transcriptional regulatory region which expresses in transformed wheat immature embryo cells at a significantly higher level than in seed tissue, and hybridizes under high stringency conditions with the rice β -glucanase gene

35 (Gns9) promoter identified by SEQ ID NO:1; (ii) a hygromycin phosphotransferase

encoding selectable marker coding sequence; and (iii) a 3' untranslated terminator region; and

(b) at least one heterologous gene expression cassette, having operatively linked in sequence in a 5' to 3' direction, (i) a transcriptional regulatory region that is expressed, induced or inducible in plant seeds, (ii) a first DNA sequence encoding a selected heterologous protein, and (iii) a 3' untranslated terminator region, wherein the transcriptional regulatory region in said heterologous-gene expression cassette is induced during seed maturation or seed germination;

culturing the wheat immature embryo cells in the presence of a selection agent effective to block growth of wheat immature embryo cells in the absence of expression of the hygromycin phosphotransferase encoding nucleic acid sequence; selecting those wheat immature embryo cells that express the hygromycin phosphotransferase enzyme, as evidenced by their growth in the presence of the selection agent; and

regenerating the selected wheat immature embryo cells into transgenic plants under non-selection conditions.

9. A wheat plant transformed by the method of claim 8.

10. A plant transformation expression cassette for transforming wheat plant cells with a chimeric selectable marker gene, said cassette comprising, in a 5' to 3' direction:

(i) a transcriptional regulatory region comprising a sequence which hybridizes under high stringency conditions with the rice β -glucanase gene promoter identified by SEQ ID NO:1, and which expresses in wheat immature embryo cells at a significantly higher level than in a selected target tissue,

(ii) a hygromycin phosphotransferase encoding selectable marker coding sequence, and

(iii) a 3' untranslated terminator region.

11. A plant transformation expression cassette according to claim 10, wherein the transcriptional regulatory region in the selectable marker gene is the Gns9 promoter identified by SEQ ID NO:1 or an operative portion thereof, said portion sufficient to promote expression in transformed wheat immature embryo cells at a significantly higher level than in a selected target tissue.

12. A wheat plant produced by regenerating a plant cell transformed with the expression cassette of claim 10.

5 13. Transgenic wheat seed comprising:

a chimeric selectable marker gene including a hygromycin phosphotransferase selectable marker coding sequence under the control of a transcriptional regulatory region that is induced in wheat immature embryo cells at a significantly higher level than in seed tissue and hybridizes under high stringency conditions with the rice β -glucanase gene (Gns9) promoter identified by SEQ ID NO:1, and

10 a heterologous protein coding sequence under the control of a transcriptional regulatory region that is induced or inducible during seed maturation or germination.

15 14. A method of transforming wheat plants with one or more heterologous nucleic acid coding sequences capable of producing heterologous proteins in the wheat, under selected induction conditions, comprising cotransforming wheat embryos with a set of two or more expression cassettes, said set comprising:

20 (a) a chimeric selectable marker expression cassette having, operatively linked in sequence in a 5' to 3' direction, (i) a transcriptional regulatory region which expresses in transformed wheat immature embryo cells at a significantly higher level than in seed tissue, and hybridizes under high stringency conditions with the rice β -glucanase gene (Gns9) promoter identified by SEQ ID NO:1; (ii) a phosphinothricin acetyltransferase (BAR) coding sequence and (iii) a 3' untranslated terminator region; and

25 (b) at least one heterologous gene expression cassette, having operatively linked in sequence in a 5' to 3' direction, (i) a transcriptional regulatory region that is expressed, induced or inducible in plant seeds, (ii) a first DNA sequence encoding a selected heterologous protein, and (iii) a 3' untranslated terminator region, wherein the transcriptional regulatory region in said heterologous-gene expression cassette is induced during seed maturation or seed germination;

30 (i) culturing the wheat immature embryo cells in the presence of a selection agent effective to block growth of the wheat immature embryo cells in the absence of expression of the phosphinothricin acetyltransferase-encoding nucleic acid sequence;

35 (ii) selecting those wheat immature embryo cells that express the phosphinothricin acetyltransferase enzyme, as evidenced by their growth in the

presence of the selection agent; and

regenerating the selected wheat immature embryo cells into transgenic plants under non-selection conditions.

5 15. A wheat plant transformed by the method of claim 14.

16. A plant transformation expression cassette for transforming wheat plant cells with a chimeric selectable marker gene, said cassette comprising, in a 5' to 3' direction:

10 (i) a transcriptional regulatory region comprising a sequence which hybridizes under high stringency conditions with the rice β -glucanase gene promoter identified by SEQ ID NO:1, and which expresses in wheat immature embryo cells at a significantly higher level than in a selected target tissue,

15 (ii) a phosphinotricin acetyltransferase-encoding selectable marker coding sequence, and

(iii) a 3' untranslated terminator region.

17. A plant transformation expression cassette according to claim 16, wherein the transcriptional regulatory region in the selectable marker gene is the Gns9 promoter identified by SEQ ID NO:1 or an operative portion thereof, said portion sufficient to promote expression in transformed immature embryo cells at a significantly higher level than in a selected target tissue.

25 *Sabal* 18. A plant transformation expression cassette according to claim 17, wherein said chimeric selectable marker gene has the sequence identified by SEQ ID NO:4.

19. A wheat plant produced by regenerating a plant cell transformed with the expression cassette of claim 17.